04-14-06 / Copy

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

OIPE APR 13 2006 PATENT & TRADEMARK OFFICE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 45

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Blaszcyk-Thurin et al
Art Unit	1653
Examiner Name	
Attorney Docket Number	WST93AUSA

Certificate APR 19 2006 of Correction

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please Identify below):

3 pp. Request for Certiificate of Correction under 35 USC Section 254

2 pp. Forms PTO/SB/44

Remarks

9 pp. Copies of Issued patent pages with errors marked in red

30 pp. Copies of specification pages, correction of filing receipt pages, and Issue fee pages. with words or phrases highlighted in blue.

Express Mail No. ED378262197US

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON		
Signature	Mary E. Bak		
Printed name	Mary E. Bak		
Date	APRIL 13, 2006	Reg. No.	31,215

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name		Date	

Customer No. 00270

APR 20 2006

WST93AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	: 09/831,047	Confirmation No.: 8220
Applicant	: Blaszcyk-Thurin et al	
Filed	: May 3, 2001	
Patent No.	: 6,960,566	
Issued	: November 1, 2005	
TC/A.U.	: 1653	
Examiner	:	
Customer No.	: 00270	
Title	: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER	

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 35 USC SECTION 254

The following errors were found in the above-identified patent:

(1) First page, Cover Page of patent, add inventor "Thomas Kieber-Emmons, Newtown Square, PA" under (75) Inventor;

(2) First page, Cover page of patent, under Assignees:, replace "Wister" with -- Wistar -- .

(3) Col. 3, line 1, replace "firther" with -- further -- ;

(4) Col. 6, line 17, replace "DLWDWWGKPAG" with -- DLWDWVVGKPAG -- ;

(5) Col. 7, line 31, replace "VGIWSWSEGSR" with -- VGIWSVVSEGSR -- ;

(6) Col. 7, line 36, replace "LAEMMG" with -- LAEMFMG -- ;

(7) Col. 17, line 67, replace "3:883" with -- 33:883 -- ;

EXPRESS MAIL NO. ED378262197US

APR 20 2006

(8) Col. 18, line 12, replace "25:1078-1082" with -- 257:1078-1082 -- ;

(9) Col. 18, line 25, replace "arc" with -- are -- ;

(10) Col. 24, line 48, replace "carbodiimidc" with -- carbodiimide -- ;

(11) Col. 27, line 10, replace "DLWDEVVGKPAG" with -- DLWDFVVGKPAG -- ;

(12) Col. 27, line 41, replace "night" with – might -- ;

(13) Col. 27, line 50, replace "DLWVDWVVDKPAG" with -- DLWDWVVGKPAG -- ;

(14) Col. 34, line 66, replace "claims to be" with -- claims are intended to be -- ;

(15) Col. 69, lines 20 and 21, Claim 1, replace, "GNCRYIGLRQPG" with -- GNCRYIGLRQFG -- ;

It is requested that a Certificate of Correction be issued to correct the above error in accordance with the enclosed Form PTO 1050, which is submitted herewith.

These errors are typographical in nature and make no substantive changes. All errors were made by the USPTO, no fee is due for correction of these errors.

Enclosed for each correction is a photocopy of the original specification page with the relevant words or phrases highlighted in blue and the corresponding original patent with errors marked in red. These documents will support the USPTO errors.

Also enclosed are copies of documents that support the correction of errors (1), (2), and (15); a description of each is listed. A copy of a Request for Correction of Filing Receipt that was filed by first class mail on May, 9, 2002 supports error (1). A copy of an issue fee that was filed by first class mail on January 25, 2005 supports error (2). A copy of a Response and Amendment that was filed by via fax on December 6, 2004 supports error (15).

The Director of the US Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

HOWSON AND HOWSON

Attorneys for the Applicants

BY Mary E. Bak

Mary E. Bak
Registration No. 31,215
501 Office Center Drive
Suite 210
Fort Washington, PA 19034
Telephone: (215) 540-9200
Telefacsimile: (215) 540-5818

PTO/SB/44 (04-05)
Approved for use through 04/30/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 6,960,566

APPLICATION NO. : 09/831,047

ISSUE DATE : November 1, 2005

INVENTOR(S) : Blaszcyk-Thurin et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(1) First page, Cover Page of patent, add inventor "Thomas Kieber-Emmons, Newtown Square, PA" under (75) Inventor;

(2) First page, Cover page of patent, under Assignees:, replace "Wister" with -- Wistar -- .

(3) Col. 3, line 1, replace "firther" with -- further -- ;

(4) Col. 6, line 17, replace "DLWDWWGKPAG" with -- DLWDWVVGKPAG -- ;

(5) Col. 7, line 31, replace "VGIWSWSEGSR" with -- VGIWSVVSEGSR -- ;

(6) Col. 7, line 36, replace "LAEMMG" with -- LAEMFMG -- ;

(7) Col. 17, line 67, replace "3:883" with -- 33:883 -- ;

(8) Col. 18, line 12, replace "25:1078-1082" with -- 257:1078-1082 -- ;

(9) Col. 18, line 25, replace "arc" with -- are -- ;

(10) Col. 24, line 48, replace "carbodiimidc" with -- carbodiimide -- ;

MAILING ADDRESS OF SENDER (Please do not use customer number below):

HOWSON AND HOWSON
Spring House Corporate Center
Box 457
Spring House, PA 19477

PTO/SB/44 (04-05)
Approved for use through 04/30/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 6,960,566

APPLICATION NO. : 09/831,047

ISSUE DATE : November 1, 2005

INVENTOR(S) : Blaszcyk-Thurin et al

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(11) Col. 27, line 10, replace "DLWDEVVGKPAG" with -- DLWDFVVGKPAG -- ;

(12) Col. 27, line 41, replace "night" with – might -- ;

(13) Col. 27, line 50, replace "DLWVDWVVDKPAG" with -- DLWDWVVGKPAG

(14) Col. 34, line 66, replace "claims to be" with -- claims are intended to be -- ;

(15) Col. 69, lines 20 and 21, Claim 1, replace, "GNCRYIGLRQPG" with -- GNCRYIGLRQFG -- ;

MAILING ADDRESS OF SENDER (Please do not use customer number below):

HOWSON AND HOWSON
Spring House Corporate Center
Box 457
Spring House, PA 19477

ERRORS(1),(2)

US006960566B1

(12) **United States Patent**
Blaszczyk-Thurin

(10) **Patent No.: US 6,960,566 B1**
(45) **Date of Patent: Nov. 1, 2005**

(54) **COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER**

(75) Inventor: **Magdalena Blaszczyk-Thurin**, Philadelphia, PA (US)

Thomas Kieber-Emmons

(73) Assignees: **The Wister Institute of Anatomy and Biology**, Philadelphia, PA (US); **The Trustees of the University of Pennsylvania**, Philadelphia, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/831,047**

(22) PCT Filed: **Nov. 5, 1999**

(86) PCT No.: **PCT/US99/26277**

§ 371 (c)(1),
(2), (4) Date: **May 3, 2001**

(87) PCT Pub. No.: **WO00/27420**

PCT Pub. Date: **May 18, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/107,478, filed on Nov. 6, 1998.

(51) **Int. Cl.**[7] **A61K 38/00**; C07K 5/00

(52) **U.S. Cl.** **514/14**; 530/300

(58) **Field of Search** 514/2, 14, 1; 530/300

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,464,935	A	*	11/1995	Heavner et al.	530/329
5,602,230	A		2/1997	Heavner et al.	
5,614,615	A	*	3/1997	Wong	536/17.9
5,618,785	A		4/1997	Heavner et al.	
5,639,734	A	*	6/1997	Esko et al.	514/25
5,643,873	A		7/1997	Barrett et al.	
5,861,505	A		1/1999	Kern	
2003/0017497	A1		1/2003	Keiber-Emmons et al.	

OTHER PUBLICATIONS

MJ Polley, ML Phillips, E Wayner, E Nudelman, AK Singhal, S Hakomori, and JC Paulson CD62 and Endothelial Cell–Leukocyte Adhesion Molecule 1 (ELAM–1) Recognize the Same Carbohydrate Ligand, Sialyl–Lewis x PNAS 1991 88: 6224–6228.*

R. Radinsky et al, "Regulation of Tumor Cell Growth at Organ Specific Metastases", in vivo, 6:325–332 (1992).

G. Nicolson, "Metastatic Tumor Cell Interactions with Endothelium, Basement Membrane and Tissue", Current Opinion in Cell Biology, 1:1009–1019 (1989).

M. Bevilacqua et al, "Endothelial Leukocyte Adhesion Molecule 1: An Inducible Receptor for Neutrophils Related to Complement Regulatory Proteins and Lectins", Science, 243:1160–1165 (Mar., 1989).

P. Brodt, "Adhesion Receptors and Proteolytic Mechanisms in Cancer Invasion and Metastasis", in Cell Adhesion and Invasion in Cancer Metastasis, ed. by P. Brodt, Chapter 11, pp. 167–242 (1996).

E. Butcher et al, "Lymphocyte Homing and Homeostasis", Science, 272:60–66 (Apr., 1996).

L. Lasky, "Selectin–Carbohydrate Interactions and the Initiation of the Inflammatory Response", Annu. Rev. Biochem., 64:113–139 (1995).

A. Varki, "Selectin Ligands", Proc. Natl. Acad. Sci. USA, 91:7390–7397 (Aug., 1994).

M. Bevilacqua et al, "Selectins", J. Clin. Invest., 91:379–387 (Feb., 1993).

G. Mannori et al, "Differential Colon Cancer Cell Adhesion to E–, P–, and L–selectin: Role of Mucin–type Glycoproteins", Cancer Research, 55:4425–4431 (Oct., 1995).

S. Karpatkin et al, "Role of Platelets in Tumor Cell Metastases", Annals of Internal Medicine, 95:636–641 (Nov., 1981).

D. Welch et al, "Tumor–elicited Polymorphonuclear Cells, in Contrast to 'Normal' Circulating Polymorphonuclear Cells, Stimulate Invasive and Metastatic Potentials of Rat Mammary Adenocarcinoma Cells", Proc. Natl. Acad. Sci. USA, 86:5859–5863 (Aug., 1989).

A. Seth et al, "T–Cell–Receptor–Independent Activation of Cytolytic Activity of Cytotoxic T Lymphocytes Mediated Through CD44 and gp90^{MEL-14}", Proc. Natl. Acad. Sci. USA, 88:7877–7881 (Sep., 1991).

F. Kolbinger et al, "The Carbohydrate–Recognition Domain of E–Selectin is Sufficient for Ligand Binding Under Both Static and Flow Conditions", Biochemistry, 35:6385–6392 (1996).

B. Revelle et al, "Single Amino Acid Residues in the E– and P–selectin Epidermal Growth Factor Domains Can Determine Carbohydrate Binding Specificity", J. Biol. Chem., 271(27):16160–16170 (Jul., 1996).

S. Li et al, "Consensus Repeat Domains of E–selectin Enhance Ligand Binding", J. Biol. Chem, 269(6):4431–4437 (Feb., 1994).

D. Erbe et al, "P– and E–Selectin Use Common Sites for Carbohydrate Ligand Recognition and Cell Adhesion", J. Cell Biol., 120(5):1227–1235 (Mar., 1993).

B. Graves et al, "Insight into E–selectin/ligand Interaction from the Crystal Structure and Mutagenesis of the lec/EGF Domains", Nature, 367:532–538 (Feb., 1994).

R. Giavazzi et al, "Rolling and Adhesion of Human Tumor Cells on Vascular Endothelium under Physiological Flow Conditions", J. Clin. Invest., 92:3038–3044 (Dec., 1993).

(Continued)

Primary Examiner—Jon Weber
(74) *Attorney, Agent, or Firm*—Howson and Howson

(57) **ABSTRACT**

Compositions containing one or more peptido-mimetics or modified peptido-mimetics of a carbohydrate ligand of an adhesion molecule in a physiologically acceptable carrier are useful for methods of reducing metastasis in a mammal and for inhibiting inflammatory response in a mammal. Particularly useful are embodiments in which the ligand is a Lewis antigen and/or the adhesion molecule is a selectin, e.g. E-selectin. Methods are disclosed for identifying peptido-mimetics of carbohydrate ligands, which may be involved in binding of tumor cells to other cells, such as endothelial cells.

4 Claims, 5 Drawing Sheets

APR 20 2006

ERROR (3)

In vivo studies have provided further evidence of the potential importance of the carbohydrate ligand/E-selectin interaction in tumor metastasis [Brodt et al., 1997, *Int. J. Cancer* 71:612–619; Mannori et al., 1997, *Am. J. Pathol.* 151:233–243 (Mannori II), Biancone et al., 1996, *J. Exp. Med.* 183:581–587].

Alternatively, some carcinoma cells do not express these carbohydrate determinants (i.e., SA-LeX and SA-Le^a) and yet they can attach to EC prior to activation. Further, this adhesion is not augmented by cytokine treatment, suggesting E-selectin-independent adhesion [Iwai et al., 1993, *Int. J. Cancer* 54:972–977, Tozeren et al., 1995, *Int. J. Cancer* 60:426–431; Miyake et al., 1992, *New Eng. J. Med.* 327:14–18; Garrigues et al., 1992, *J. Cell. Biol.* 125:129–142].

Studies have also demonstrated the role of oligosaccharides in inflammatory responses. Neutrophil extravasation is enabled by a multistep process initiated by the selectin family [Kansas, 1996, *Blood* 88: 3259–3287]. Neutrophil-endothelial cell interaction mediated via the selectins in the context of vascular shear flow, are characterized by transient tethering of the neutrophils, followed by rolling of the neutrophil along the endothelial surface of the vessel wall. Studies in vivo and in vitro indicate that selectin-dependent neutrophil rolling is essential to subsequent events in the transmigration process. Neutrophils are exposed to endothelial cell derived IL-8, platelet-activating factor and other neutrophil-activating molecules [Lowe, 1997, In: *The selectins: Inhibitors of leukocyte endothelial adhesion*, pp. 143–177, Vestweber, ed., Harwood Academic Publishers, Reading, UK], which in turn promote activation of neutrophil P2 integrins, leading to integrin-dependent firm adhesion to the integrin receptor ICAM-1, and finally to neutrophil extravasation, possibly via homophilic interaction of platelet/endothelial cell adhesion molecule 1.

The expression of ligands for selectins, particularly E-selectin, by both neutrophils and carcinoma cells raises the possibility that metastases are equivalent to the inflammatory process in which tumor cells, particularly carcinoma cells, use the same molecular mechanism(s) for cancer cell-EC interaction as lymphocytes, through the adhesion interaction of the endothelial cell selectins with the tumor-associated carbohydrate ligands, e.g., SA-LeX, SA-Le^a, and LeY.

In addition to their role in cell adhesion, carbohydrate structures also play a role in angiogenesis. Aberrant angiogenesis can occur in a variety of pathologic conditions. Neovascularization of tumors occurs by aberrant stimulation of normally quiescent endothelial cells to migrate, proliferate and form new capillary blood vessels [Ingber and Folkman, 1989, *J. Cell. Biol.* 109:3317–3330]. The experimental evidence suggests that E-selectin and its ligand SA-LeX function in angiogenesis [Nguyen et al., 1982, *J. Biol. Chem.* 267:26157–26165]. Thus, proliferating microvascular endothelium presents a potential target for anti-cancer and anti-angiogenic therapies through the inhibition of E-selectin-dependent carbohydrate-mediated interactions [Folkman, 1995, *N. Engl. J. Med.* 333:1757–1763].

Although recent studies suggest the importance of carbohydrate ligand-cell adhesion interactions in tumor metastasis, angiogenesis and inflammatory responses, the complex nature of the carbohydrate ligands involved has long hampered studies of these processes. The difficult chemical or enzymatic synthesis required by these complex carbohydrate ligands and the technical complexity involved in analyzing the functional/structural interactions of these ligands with selectins at the molecular level have severely hindered the development of anti-adhesion therapeutics for treatment of these disease processes for which there is no effective treatment.

Many peptide mimics of carbohydrate structures have been described in the literature [see, e.g., Agadjanyan, M. et al, 1997, *Nature Biotechnol.* 15: 547–551, among others] including those binding with high affinity to E-selectin [Tsukida, T. et al, 1998, *J. Med. Chem.* 41: 4279–4287]. A peptide that mimics the GD1 ganglioside, also involved in cell adhesion and metastasis of melanoma cells, has been recently described [Ishikawa, D. et al, 1998, *FEBS Lett.* 441: 20–24]. This peptide isolated from a peptide phage display library using an anti-GD1 antibody inhibits metastasis in an in vivo model.

Thus, there remains a long-felt and acute need for the development of techniques and probes for the study of complex carbohydrate ligand-cell adhesion molecule interactions, and for the development of anti-tumor, anti-inflammatory and angiogenesis-blocking therapeutics based on the selective inhibition of these interactions.

SUMMARY OF THE INVENTION

The present invention meets the above-stated needs by providing novel peptido-mimetics of carbohydrate structures and uses therefor in affecting animal cell adhesion mediated by carbohydrate ligand-cellular lectin protein receptor interactions.

In one aspect, the invention includes a composition comprising a peptido-mimetic of a carbohydrate ligand of an adhesion molecule in a physiologically acceptable carrier. In one embodiment, the adhesion molecule is a selectin, particularly E-selectin. In another embodiment, the ligand is a Lewis antigen. Particularly desirable are peptidomimetics of the Lewis antigens SA-Le^a, SA-LeX, and LeY. A variety of specific peptido-mimetics are recited in the detailed disclosure such peptido-mimetics may be modified as described herein.

In still another aspect, the invention provides a method of modulating binding of an adhesion molecule to a carbohydrate ligand. The method comprises contacting the adhesion molecule (e.g., a selectin) with a peptido-mimetic of the carbohydrate ligand, so that binding of the adhesion molecule to the carbohydrate ligand is modified or altered in a therapeutically effective manner.

In a further aspect, the invention provides a method of modulating adhesion of a tumor cell to an adhesion molecule located on an endothelial cell. The method comprises contacting the tumor cell with a peptido-mimetic of a carbohydrate ligand. The peptido-mimetic modulates adhesion of the tumor cell to the endothelial cell. The adhesion molecule may be, e.g., a selectin. The ligand is preferably a Lewis antigen.

In still another aspect, the invention provides a method of treating cancer in a mammal by administering an effective amount of a peptido-mimetic of a carbohydrate ligand to the mammal. The ligand is preferably a Lewis antigen. Administration of the peptido-mimetic reduces adhesion of tumor cells to endothelial cells in the mammal, thereby reducing metastasis of the cancer.

In yet a further aspect, the invention provides a method of inhibiting an inflammatory response in a mammal by contacting an endothelial cell with an effective amount of a peptido-mimetic of a carbohydrate ligand. The ligand is preferably a Lewis antigen. The specific peptido-mimetics described herein can be used in this method.

ERROR (4)

As another aspect, the invention provides additional methods of identifying a variety of peptido-mimetics of carbohydrate ligands. The peptido-mimetics inhibit the normal binding between the ligand and its natural binding partner. In one embodiment, the binding partner is an adhesion molecule, such as a selectin. In another embodiment, carbohydrate ligand is located on the surface of a tumor cell and is a Lewis antigen. In a further embodiment of this method, the carbohydrate ligand is located on the surface of a tumor cell and it normally binds to an adhesion molecule on human umbilical cord vein endothelial cells (HUVEC). In another embodiment, the carbohydrate ligand affects capillary tube formation or angiogenesis. In still another embodiment, the peptido-mimetic affects adhesion of a selected cell, e.g., a neutrophil or tumor cell, to an adhesion molecule located on an endothelial cell. In another embodiment, the carbohydrate ligand affects neutrophil recruitment. The steps of these methods are described in detail below.

In yet another aspect, the invention provides a method of producing peptido-mimetics of Lewis antigens, particularly peptido-mimetics not including APWLYAGP [SEQ ID NO: 83]. This method comprises the steps of screening a random peptide library, in which the peptides are expressed as fusion proteins on the surface of bacterial clones, with antibodies specific for the Lewis antigens and/or an E-selectin immunoglobulin fusion protein; and selecting clones which bind the antibodies and/or the fusion protein. The selected clones produce peptido-mimetics of the Lewis antigens.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a diagram depicting the reduction of neutrophil influx upon administration of DLWDWVVGKPAG [SEQ ID NO: 1] mimicking SA-Le^a carbohydrate in mice with chemically induced peritonitis. The neutrophil count results were obtained from four experiments (three mice in each group). Unrelated peptide was administered in control mice. Statistical analysis of the data using nonparametric unpaired t-test yielded P values <0.001 for the data shown in FIG. 1A.

FIG. 1B is a diagram illustrating the myeloperoxidase activity in the collected neutrophils of the animals of FIG. 1A. Statistical analysis of the data using nonparametric unpaired t-test yielded p values <0.005 for this data.

FIG. 2A is a bar graph demonstrating the E-selectin-independent adhesion of human mammary adenocarcinoma cells SkBr5 to the human endothelioma cell line ECV-304. SkBr5 and HUVEC cells were allowed to adhere for 15 minutes in the continuous presence of MAbs and MAb $F(ab)_2$ fragments (marked in the graph) at 40 pg/ml. Control anti-influenza hemagglutinin MAb H24135 was used at the same concentration. The results represent the percentage (%) of adherent cells in the presence of specific MAb as compared to control anti-influenza hemagglutinin MAb H24135. Results are reported as means±standard error (SE) of five independent experiments.

FIG. 2B is a graph depicting the representative data of the expression of various antigens on the surface of cells in the absence (−) or presence (+) of the inflammatory cytokine, Interleukin-1, as determined by MAbs binding in radioimmunoassay (RIA) with monolayer ECV-304 cells. The presence of the following antigens was detected using the MAbs indicated: SA-Le^a(MAbNS19-9), CD63 (MAb C029 and ME491), E-selectin (specific MAb anti-ELAM-1, British Bio-Technology, UK), and anti-influenza virus hemagglutinin negative control (MAb H24135).

FIG. 3 is a graph illustrating the effect of Trp5 substitution with Phe in peptide DLWDWVVGKPAG [SEQ ID NO: 1] resulting in peptide DLWDFVVGKPAG [SEQ ID NO: 63] on binding of SA-Le^a specific MAb NS19-9. Constant amounts of Mab were incubated with increasing amounts of peptides and binding of free antibody to carbohydrate SA-Le^a was measured by enzyme linked immunosorbent assay. Results show competitive inhibition of MAb binding to solid phase SA-Le^a polyacrylamide matrix (SA-Le^a-PAA) by 12-mer peptides DLWDWVVGKPAG (■) [SEQ ID NO: 1] and DLWDFVVGKPAG (▲) [SEQ ID NO: 63] with respect to the MAb binding without peptide (100% of binding) and a negative control unrelated peptide (○).

FIG. 4 is a circular dichroism (CD) spectra comparing dodecapeptides DLWDWWGKPAG (solid line) [SEQ ID NO: 1] and DLWDFVVGKPAG (---) [SEQ ID NO:63]. The spectra were recorded at 0.51 mg/ml for both peptides.

FIG. 5 is a graph illustrating the inhibition of lung experimental metastases with peptide DLWDFVVGKPAG [SEQ ID NO: 63]. Tumor cells were admixed with the specific or unrelated peptide solution (1 mg per mouse) and animals were inoculated with 1×10^5 B16F10FtIII tumor cells in 200 μl volume of PBS via tail vein. Results are from 4 experiments (5 mice in each group) are shown. Each dot represents enumerated tumor nodules in one lung in experimental group of C57B1/6 mice treated with the peptide (panel B), control group of C57B1/6 mice treated with unrelated peptide (panel A) and E-selectin knock-out (KO) mice of C57B1/6 background (panel C). Statistical analysis using a nonparametric unpaired t test gave a two-tailed p values <0.008 and 0.009 for animals treated with peptide and E-selectin KO, respectively, as compared to control group. The horizontal bars represent median values and vertical bars denote standard deviation.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the discovery that peptido-mimetics of complex carbohydrate structures block adhesion of tumor cells and leukocytes to endothelial cells. The production of small peptide molecules which mimic complex carbohydrate structures are useful for blocking carbohydrate ligand-cell adhesion molecule interactions involved in metastasis, angiogenesis, and inflammatory responses. Thus, these peptido-mimetics are useful as anti-adhesion therapeutics. The evaluation of complex carbohydrate ligand-adhesion molecule interactions at the molecular level enables the design of peptido-mimetics and possibly other compounds which block these adhesion interactions thereby disrupting the disease processes mediated by them. Further, the ability to generate peptide mimotopes of complex carbohydrate structures enables the use of recombinant peptide library display technology in discovering novel blocking agents for important therapeutic targets which bind carbohydrate ligands. The compositions and methods of this invention are also useful in designing additional therapeutics to inhibit the adhesion interactions required for disease processes.

As used herein, the term "peptido-mimetic" means a peptide or polypeptide that mimics complex carbohydrate conformations and structures. A subset of peptido-mimetics are referred to as "mimotopes". Mimotopes are small peptido-mimetics, generally from 7 to about 15 amino acids in length which mimic complex carbohydrate structures,

ERROR (5), (6)

including the carbohydrate ligands for endothelial adhesion molecules. Mimotypes are also capable of blocking the ligand-adhesion molecule interaction. Throughout this specification, these term are used interchangeably. These small peptides also facilitate the study of complex structural/conformational relationships between these ligands and cellular lectins.

A. Peptides of the Invention

A composition of this invention comprises at least one peptido-mimetic of a carbohydrate ligand of an adhesion molecule in a physiologically acceptable carrier. In one embodiment, the adhesion molecule is a selectin, such as E-selectin. In another embodiment, the ligand is a Lewis antigen. Particularly desirable are peptido-mimetics of the Lewis antigens SA-Le^a, SA-LeX, and LeY. The illustrative peptides described below may be modified, as described in more detail herein, to improve anti-adhesion properties and to increase their stability to degradation in vivo.

Desirable compositions of this invention contain one or more of the following peptido-mimetics which mimic the topography of the E-selectin ligand: ASAVNLYIPTQE [SEQ ID NO:84], VYLAPGRISRDY [SEQ ID NO:85], VYLAPGRFSRDY [SEQ ID NO:86], CTSHWGVLSQRR [SEQ ID NO:87], RVLSPESYLGPS [SEQ ID NO:88], RVLSPESYLGPA [SEQ ID NO:89], VGNGVLMGRRG [SEQ ID NO:90], RVLSPESYLGPA [SEQ ID NO:92], GNCRYIGLRQFG [SEQ ID NO:93], DIRVEPGGGYTH [SEQ ID NO:94], APIHTYTGRARG [SEQ ID NO:96], and RHTCVRSCGHDR [SEQ ID NO:97].

Similarly, exemplary peptido-mimetics of SA-Le^a include, without limitation, VGIWSWSEGSR [SEQ ID NO: 102], RCSVGVPFTMES [SEQ ID NO:103], QDGVWEHVLEGG, [SEQ ID NO:104], DLWDWV-VGKPAG [SEQ ID NO:1], VELSGRGGLCTW [SEQ ID NO:105], VIGAASHDEDVD [SEQ ID NO:106], TIEPV-LAEMMG [SEQ ID NO:107], DKETFELGLFDR [SEQ ID NO:108], FSGVRGVYESRT [SEQ ID NO:109], PDDAP-MHSTRVE [SEQ ID NO:110], STGLMVDFLEPG [SEQ ID NO: 91], AKTFGLEHGCEA [SEQ ID NO: 95], GGTVEVWSIKGG [SEQ ID NO: 115], DHFSQAGSS-NHH [SEQ ID NO: 116]; DDPVTPVIDFGK [SEQ ID NO: 117], and RDGLIDFVVAGT [SEQ ID NO: 118].

As described in the examples below, families of mimics of carcinoma-associated antigens that represent SA-Le^a, in particular, were identified from a combinatorial peptide library using MAb NS 19-9 specific for this carbohydrate structure. One of the peptides, DLWDWVVGKPAG [SEQ ID NO: 1], was selected that specifically competes for binding of MAb for SA-Le^a. This peptide displays an ability to partially inhibit neutrophil recruitment in an acute inflammation model in vivo. As described below, this peptide was analyzed by systematic amino acid replacements to identify optimal conformationally stabilized SA-Le^a mimics with higher affinity using a solid phase peptide array library. Comparison of signal intensities revealed significant differences in MAb binding as a result of substitutions, in particular at the N-terminus. Substitution analysis allowed for delineation of key residues that were sensitive to replacement. MAb NS19-9 discriminated against multiple amino acid substitutions affecting its recognition. Specific residues within this peptide were identified that may contribute to the mimicry of carbohydrate structure by the peptide.

On the other hand MAb NS19-9 could tolerate replacement of the lead peptide sequence by a variety of amino acids. These substitutions did not abrogate binding, suggesting that they did not affect the structural specificity required for MAb recognition. Different amino acids in themselves can act as structural mimics within an identified peptide and bind through non-specific interactions. The different consensus sequences among the families of peptides identified with the same MAb from the random peptide library or sequences without an obvious consensus characterized in previous studies support the notion that indeed different amino acid residues provide structural similarity. Alternatively, different consensus sequences mimic different topographies of the carbohydrate epitope recognized by the antibody.

The identification of several cross-reactive peptides displaying higher NS19-9 binding further delineate specific residues that may improve upon peptide mimicry of the carbohydrate structure. Several substitutions within the C-terminus, in particular with amino acids containing carboxyl groups that increased MAb binding, were identified suggesting an important role of polar interactions in binding affinity. The strongest signal however resulted from the single amino acid substitution of Phe for the Trp at position 5, creating the SA-Le^a peptidomimetic DLWDFVVGKPAG [SEQ ID NO: 63].

The present invention demonstrates that peptides mimicking SA-Le^a are able to bind surfaces of proteins specific for these structures and thus they can act as antagonists for the recognition of the cognate carbohydrate antigen or ligand. In vivo oligosaccharide dependent reduction of metastasis formation may be a function of the interruption of these interactions. Antagonists interfere with the metastatic process at the level of cellular adhesion and blood vessel formation, since E-selectin and SA-LeX are expressed on actively growing blood vessels. Alternatively, peptide mimics act on signal transduction events mediated by selectins and their ligands and the in vivo consequences of selectin-ligand antagonism to the complex signal transduction processes associated with selectin-dependent cell adhesion.

Certain exemplary peptidomimetics of LeY include the peptides TKRPDLIVDPIP [SEQ ID NO:98], DEVRP-DLISTEE [SEQ ID NO: 99], NLRPKYIXLDAD [SEQ ID NO:100), and TLIAFADLVDVI [SEQ ID NO: 101].

Peptides mimicking carbohydrate antigens retain conformational properties of cognate carbohydrate structures and can block recognition of cells expressing such ligands in vivo. Thereby, they can mediate anti-metastatic functions as demonstrated by blocking of experimental metastasis. Thus, the peptides identified above which mimic the topography of the E-selectin ligand and/or the other Lewis antigens can be employed in pharmaceutical compositions. Such peptido-mimetics are useful in pharmaceutical compositions directed toward the treatment of cancer, and the prevention and/or inhibition of metastases (see, e.g., Example 14). Such peptides may also be used in pharmaceutical treatments to block selectin-dependent interactions, e.g. diminishing the inflammatory response (see, e.g., Example 8).

The above peptido-mimetics and others which may be identified by use of the assays referred to below may be further modified to increase or enhance the stability of such peptides for in vivo use or to enhance the binding abilities of these peptido-mimetics. Peptido-mimetics according to this invention includes peptido-mimetics such as those identified specifically above, which are modified to increase their stability in vivo.

For example, the incorporation of unnatural amino acids (e.g., D configuration amino acids) at the N or C termini, the most frequent peptide degradation sites, may improve the pharmacological properties of the peptides, without loss of

ERRORS (7), (8), (9)

be computationally evaluated and designed by means of a series of steps in which chemical entities or fragments are screened and selected for their ability to associate with the peptides of this invention.

Specialized computer programs that may also assist in the process of selecting fragments or chemical entities similar to the peptides, or entities which can interact with the peptides and thus mimic the receptor, include the GRID program available from Oxford University, Oxford, UK. [P. J. Goodford, "*A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules*", *J. Med. Chem.*, 28:49–857 (1985)]; the MCSS program available from Molecular Simulations, Burlington, Mass. [A. Miranker and M. Karplus, "*Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method*", *Proteins: Structure, Function and Genetics*, 11:29–34 (1991)]; the AUTODOCK program available from Scripps Research Institute, La Jolla, Calif. [D. S. Goodsell and A. J. Olsen, "*Automated Docking of Substrates to Proteins by Simulated Annealing*", *Proteins: Structure, Function, and Genetics*, 8:195–202 (1990)]; and the DOCK program available from University of California, San Francisco, Calif. [I. D. Kuntz et al, "*A Geometric Approach to Macromolecule-Ligand Interactions*", *J. Mol. Biol.*, 161:269–288 (1982)], software such as Quanta and Sybyl, followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER. Additional commercially available computer databases for small molecular compounds include Cambridge Structural Database, Fine Chemical Database, and CONCORD database [for a review see Rusinko, A., *Chem. Des. Auto. News*, 8:44–47 (1993)].

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound. Assembly may proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure of the peptido-mimetic of this invention. Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include the CAVEAT program [P. A. Bartlett et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in *Molecular Recognition in Chemical and Biological Problems*", Special Pub., Royal Chem. Soc. 78, pp. 182–196 (1989)], which is available from the University of California, Berkeley, Calif.; 3D Database systems such as MACCS-3D database (MDL Information Systems, San Leandro, Calif.) [see, e.g., Y. C. Martin, "3D Database Searching in Drug Design", *J. Med. Chem.*, 35:2145–2154 (1992)]; and the HOOK program, available from Molecular Simulations, Burlington, Mass.

Compounds that mimic a peptide of this invention or a ligand of the peptides may be designed as a whole or "de novo" using either an empty active site or optionally including some portion(s) of a known ligand(s). Suitable methods describing such methods include the LUDI program [H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", *J. Comp. Aid. Molec. Design*, 6:61–78 (1992)], available from Biosym Technologies, San Diego, Calif.; the LEGEND program [Y. Nishibata and A. Itai, *Tetrahedron*, 47:8985 (1991)], available from Molecular Simulations, Burlington, Mass.; and the LeapFrog program, available from Tripos Associates, St. Louis, Mo. Other molecular modelling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al, "Molecular Modeling Software and Methods for Medicinal Chemistry", *J. Med. Chem.*, 3:883–894 (1990). See also, M. A. Navia and M. A. Murcko, "The Use of Structural Information in Drug Design", *Current Opinions in Structural Biology*, 2:202–210 (1992). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the peptide of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, e.g., P. S. Farmer, *Drug Design*, Ariens, E. J., ed., Vol. 10, pp 119–143 (Academic Press, New York, 1980); U.S. Pat. No. 5,331,573; U.S. Pat. No. 5,500,807, C. Verlinde, *Structure*, 2:577–587 (1994); and I. D. Kuntz, *Science*, 25:1078–1082 (1992). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Thus, using these computer evaluation systems, a large number of compounds may be quickly and easily examined and expensive and lengthy biochemical testing avoided. Moreover, the need for actual syntheses of many compounds is effectively eliminated. Once identified by the modelling techniques, the proposed "new antibacterial" compound may be tested for bioactivity using standard techniques, such as the assays of the examples below.

The invention is further described in detail by reference to the following, experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLE 1

Screening of a Random Peptide Library

To develop novel molecules to inhibit the adhesion of human adenocarcinoma cells to EC and, ultimately, to inhibit metastasis in vivo, peptides were derived from a 12-mer random peptide library. A diverse library of random dodecapeptides, displayed as flagellin-thioredoxin fusion proteins (FLITRX) on the surfaces of *E. Coli* bacterial cells, was obtained from Invitrogen (Carlsbad, Calif.) [LaVallie et. al., 1993, *Bio/Technology*, 11:187–193]. This library enables efficient isolation of bacteria displaying peptides with affinity to immobilized antibodies or to other binding proteins. The use of this library offered advantages over phage display in which the level of expression of phage coat protein genes is low and the selected peptides are usually unconstrained molecules with many degrees of conformational freedom. Moreover, no phage infection or isolation steps are necessary using, the FLITRX fusion protein system. Such highly diverse peptide libraries offer many distinct advantages over difficult chemical or enzymatic synthesis of complex carbohydrates, providing for the inexpensive and rapid identification and optimization of novel ligands.

Specifically, an aliquot of the FLITRX library containing at least 2×10^{10} cells to ensure full representation of peptides, was grown to saturation for 15 hours in IMC/amp 100 medium (M9 medium containing 1 mM $MgCl_2$ supplemented with 0.5% glucose, 0.2% casamino acids and 100 μg/ml ampicillin). The expression of thioredoxin with incorporated 12-mer peptide sequence was induced by further 6 hours of incubation of a culture of 10^{10} cells, diluted 1:25 with fresh IMC/amp 100 medium containing 100 μg/ml tryptophan. The induced bacteria were panned on a MAb-coated tissue culture (20 μg/ml) plate followed by blocking with 1% nonfat milk containing 150 mM NaCl and 1%

ERROR (10)

DESIGN (LUDI) program (Biosym Technologies, San Diego, Calif.) [Bohm 1992, *J. Comput. Aided Mol. Des.* 6:593–606]. This program searches a molecular library for fragments representative of the amino acids in the target peptide sequence. The program then positions the fragments within the combining site devoid of steric conflicts.

Previously, a molecular basis for interaction of LeY tetrasaccharide and MAb BR55-2 binding site was elucidated using molecular modeling [Blaszczyk-Thurin et al., 1996, *Protein Engineering* 9:101–113]. Modeling studies of the APWLYAGP [SEQ ID NO: 83] sequence mimicking LeY carbohydrate in the combining site of the anti-LeY antibody B3 [Murali and Kieber-Emmons, 1997, *J. Molec. Rec.*, 10:269–276] indicate that the putative contact residues APWLYA of SEQ ID NO: 83 adopt a turn conformation. Such a conformation is also projected for residues displayed in the constrained library.

In order to establish how the putative APWLY sequence of SEQ ID NO: 83 mimics LeY binding to B3, the pentapeptide sequence was "fitted" into the B3 combining site using the LUDI program. Using this program, the APWLY sequence of SEQ ID NO: 83 was modeled such that the Trp (W), Tyr (Y), Leu (L) and Ala (A) residues occupied relative positions as the identified LUDI fragments. Judicious positioning relied upon intermolecular interaction calculations in which several potential binding modes of the peptide were ranked according to the stability of the complex.

In the most stable conformation, the AP residues occupied a similar position to the LeY GlcNAc residue. This positioning indicates that the proline residue mimics the spatial position of the glucose unit of GlcNAc, while the Ala methyl group is positioned similarly to the terminal methyl group of GlcNAc's N-acetyl. The Trp residue occupies a volume associated with the Fuca 1,3 moiety, and the Leu residue occupies the volume of the β Gal moiety and has the hydrophobic interaction of β Gal. The Tyr residue occupies a position not associated with LeY binding to B3. The computer modeling disclosed that the low energy binding mode conformation adopts a turn region similar to that observed for the YPY motif in binding to ConA [Kaur et al., 1997, *J. Biol. Chem.* 272:5539–5543]. This conformation lends itself to the Tyr residue of the peptide to potentially interact with several residues in CDR2 of the heavy chain of B3 that include Asp H53, Ser H52, Ser H55, or Ser H56. These residues are different in BR55-2, which does not bind the monovalent APWLYGPA [SEQ ID NO: 121] peptide in a series of ELISA assays.

More importantly, energy optimization of the positioned peptide identified similar functional groups within the B3 combining site in contact with the peptide and carbohydrate tetrasaccharide core of LeY. Therefore, this analysis provides a strategy for determining the molecular basis for antigenic mimicry of particular motifs, providing a unique perspective of how a peptide sequence fits into the antibody or a receptor combining site, competing with a native antigen. This approach also enables the design of therapeutic compounds which more effectively compete with the native carbohydrate ligand for binding to cell adhesion molecules.

The above-disclosed approach was further extended to determine the types of motifs that bind to BR55-2 and whether such motifs could be isolated with BR55-2 from a peptide phage screen. Identification of such peptides as motifs would be a first step in to improving upon antigenic mimicry for LeY. Search of the LUDI database using the modeled structure of BR55-2 identified the motifs YPY, YRY and WRY, which are known to mimic various carbohydrate subunits, as interacting with BR55-2 and also identified a non planar-X-planar type motif, FSLLW [SEQ ID NO: 114], as possibly interacting. Thus, non-overlapping residue types were identified using the LUDI program as in the B3 studies. A computer-generated space filling model was generated which depicted the identification and placement of an optimized "FSLLW" amino acid motif in the combining site of anti-LeY monoclonal antibody BR55-2 in contrast to LeY positioned in the BR55-2 combining site. The topological similarity is very good. This peptide competes with LeY for BR55-2 binding. These data further demonstrate another method to develop peptide mimotopes that are specific. This method may be extended to identify motifs mimicking SA-LeX and SA-Le^a using the crystal structure of the lectin domain of E-selectin in optimizing respective mimotopes.

EXAMPLE 6

Identification of Sequences Critical for MAb Binding and an SA-Le^a Mimic with Higher Antibody Binding Affinity

To analyze amino acid residues that are critical for NS19-9 recognition, an array library of 163 unique peptides was generated by systematic amino acid replacement in which each position of the starting peptide DLWDWV-VGKPAG [SEQ ID NO: 1] was replaced by other L-amino acids. In addition, peptides were synthesized with simultaneous incorporation of multiple amino acids or with truncation of specific regions.

The peptide array of 163 unique peptides was generated by substituting all amino acids for each individual amino acid in lead peptide (DLWDWVVGKPAG [SEQ ID NO: 1] identified by combinatorial library panning with MAb NS19-9. An array of synthetic 12-mer peptides was synthesized using 90×130 mm polyethylene glycol-modified cellulose membrane functionalized with approximately 4 nmole/mm^2 amino groups, manufactured by Abimed (Lagenfeld, Germany). Standard Fmoc chemistry was used according to the manufacturer's instructions [Frank, R., 1992, *Tetrahedron* 48, 9217–9332]. The protected and activated amino acids were spotted using an Abimed ASP 422 robotic arm. All washing, dyeing and deprotection steps were done manually. The activated C-terminal amino acids were spotted leaving 10 mm space in each direction, at the concentration of 0.5 M in N-methyl pyrrolidone. A volume of 0.5 ml provides spot of 7–8 mm in diameter. Activation of the amino acids with dicyclohexyl-carbodiimide and N-hydroxy-benzotriazole was done 30 minutes before spotting. After each coupling cycle, the paper was washed with 12% acetic anhydride dissolved in N,N'-dimethylformamide (DMF) twice for a total of 10 min to endcap all unreacted amino groups. Repetitive removal of the Fmoc groups was achieved by two treatments with 20% piperidine in DMF for 5 and 10 min, respectively. The second and consecutive amino acids were coupled in a 1.1 molar excess, and were spotted 3–4 times depending upon the outcome of the bromophenol blue assay of the couplings.

After the coupling and deprotection steps, the membrane was washed thoroughly with DMF and ethanol, dried and stained with bromophenol blue dissolved in DMF. After successful coupling the paper remains colorless; after successful deprotection steps the peptide dots turn deep blue. The coupling steps were repeated until all peptide spots remained colorless. The N-terminal amino acids at the end of the syntheses remained uncapped. Final removal of the side-chain protecting groups was performed by washing the

ERRORS (11), (12) (13)

Array analysis failed to reveal significant differences in the binding intensities between the peptides substituted at different positions, suggesting that single substitution at any position in this region with carboxyl groups can enhance the interaction with the MAb binding site. In addition, substitutions with Ile, Ala and Ser also improved MAb binding. Similarly, the simultaneous replacement of several residues with clusters of amino acids upstream from position 6 demonstrated enhanced binding. The highest intensity signal was, however, observed with peptide DLWDEVVGKPAG [SEQ ID NO:63] containing a single substitution at position 5 with Phe.

Table 5 lists the amino acid substitutions within peptide DLWDWVVGKPAG [SEQ ID NO: 1] that increase the binding of MAb NS19-9 using the peptide array.

TABLE 5

Peptide Sequence	SEQ ID NO
DLWDFVVGKPAG	63
DLWDWVIGKPAG	64
DLWDWVVAKPAG	65
DLWDWVVSKPAG	66
DLWDWVVEKPAG	67
DLWDWVVDKPAG	68
DLWDWVVGEPAG	69
DLWDWVVGDPAG	70
DLWDWVVGKEAG	71
DLWDWVVGKDAG	72
DLWDWVVGKPDG	73
DLWDWVVGKPAD	74
DLWDWVKEKPAG	75
DLWDWVLAKPAG	76
DLWDWVVGEDAG	77
DLWDWVVGKPEK	78
DLWDWVKEEPAG	79
DLWDWVVGKDEK	80
DLWDWVVGEDEK	81
DLWDWVKEEDEK	82

A distinct pattern of substitutions that led to increased or abolished signal intensities with respect to the C- and N-terminus suggests that the region close to the N-terminus might contribute to the specificity of the interaction with NS19-9. Amino acids close to the C-terminus appear to add significantly to the affinity of ligand binding.

EXAMPLE 7

Inhibition of Binding of MAb to the Carbohydrate with Synthetic Peptides Mimicking SA-Le^a Structure

DLWVDWVVGKPAG [SEQ ID NO:1] and DLWDFVVGKPAG [SEQ ID NO:63] were chemically synthesized and tested for their ability to compete to immobilized synthetic SA-Le^a-PAA neoglycoprotein. To determine whether peptide SEQ ID NO: 1 and peptide SEQ ID NO: 63 are true mimics of SA-Le^a, dose-response experiments were carried out in order to determine the concentration of peptides required for blocking of 50% of MAb binding to the native carbohydrate antigen (IC_{50}), as determined by competition ELISA (Example 3). Both peptides SEQ ID NOS: 1 and 63 blocked the binding of MAb NS 19-9 to the constant amount of carbohydrate antigen in a dose-dependent manner. The IC_{50} for peptide SEQ ID NO: 1 blocking of MAb-SA-Le^a binding was 700 μM. Peptide SEQ ID NO: 63 exhibited a more pronounced dose-dependent inhibition of the MAb-SA-Le^a binding as compared with peptide SEQ ID NO: 1 as demonstrated by the calculated IC_{50} value of 70 μM for peptide SEQ ID NO: 63.

Without wishing to be bound by theory, these data suggest that the peptide sterically interferes with MAb binding to carbohydrate antigen. The sequences DLWDWVVGKPAG [SEQ ID NO: 1] and DLWDFVVGKPAG [SEQ ID NO:63] represent solvent-accessible epitopes and the peptides represent cognate determinants for the antibody. No measurable blocking of anti-Le^a MAb NS 19-9 binding was found with non-related peptide, indicating that the inhibitory effects of the native sequences are due to specific effects.

EXAMPLE 8

Inhibition of Neutrophil Recruitment in an Acute Inflammation Model In Vivo by a Peptido-Mimetic of SA-Le^a

The accumulation of neutrophils is a characteristic feature of acute and chronic inflammatory disease, and early steps in the recruitment of these cells to the site of inflammation depends upon E-selectin-mediated interaction. Thus, inhibition of neutrophil recruitment in vivo is an important test of the ability of potential therapeutic agents to inhibit E-selectin-mediated events. SA-Le^a interaction with E-selectin is not relevant for adhesion of neutrophils, since neutrophils do not express SA-Le^a on their surfaces. However, tumor cells have been demonstrated to prefer SA-Le^a over SA-LeX in mediating E-selectin-dependent adhesion interactions with endothelial cells. Because SA-Le^a binds to E-selectin, the adhesion-blocking ability of an SA-Le^a peptido-mimetic was measured in a neutrophil-recruitment in vivo model of acute inflammation. Thus, this assay demonstrates that the administration of a SA-Le^a mimicking molecule inhibits neutrophil recruitment, i.e., diminishes the influx of neutrophils into chemically irritated peritoneum in vivo.

The bioactivity of the 12-mer peptide SEQ ID NO: 1 which mimics the SA-Le^a carbohydrate structure was determined by administering Zymosan™ intraperitoneally (i.p.) into mice [see, e.g., Martens et al. 1995, *J. Biol. Chem.* 270:21129–21136; Rao et al. 1994, *J. Biol. Chem.* 269:19663–19666], followed three hours later by an intravenous (i.v.) injection of peptide SEQ ID NO: 1 (1 mg). Neutrophils were harvested and counted one hour later. As shown in FIG. 1A, peptide treatment significantly ($P<0.001$) reduced the number of neutrophils in peritoneal lavage fluids. Control experiments using the same dose of "scrambled" peptide sequence, which did not bind to NS19-9 MAb, exhibited no decrease in neutrophil influx relative to PBS-injected mice.

To confirm these results, myeloperoxidase (MPO), activity, which is an enzymatic marker for neutrophils, was measured spectrophotometrically as an absorbance rate in the homogenates of collected cells [Bradley et al., 1982, *J. Invest. Derm.* 78:206–209]. Significant reduction of enzymatic activity ($P<0.005$) was observed in parallel with decreased neutrophil numbers assessed by total neutrophil count (FIG. 1B). The reduction in enzyme activity is apparently due to reduction in neutrophil recruitment in mice treated with peptide mimicking E-selectin ligand SA-Le^a. This peptide inhibits E-selectin function in vivo. Although peptide SEQ ID NO: 1 exhibited relatively low-affinity binding to MAb in vitro, IC_{50} data from in vitro competition assays (Example 3) may not be relevant to the in vivo situation since the E-selectin blocking effect was clearly observed despite the low affinity demonstrated in vitro.

EXAMPLE 9

Peptido-Mimetics of E-Selectin Ligand

To identify peptides mimicking E-selectin ligand that may involve other than carbohydrate epitopes of the natural

ERROR (15)

in multiple in vitro studies. An in vivo experimental metastatic model which permits investigation of SA-Le^a supported adhesion of tumor cells to lung endothelium is performed as follows:

B16F10 murine melanoma cells do not naturally express E-selectin ligands SA-LeX or SA-Le^a as demonstrated by FACS analysis, and are syngeneic with C57B1/6 haplotype (American Type Tissue Collection, Rockville, Md.). To manipulate these cells to express the SA-Le^a structure on the tumor cell surface, the B16F10 cells were stably transfected with pCDNA-FTIII using Effectene (Qiagen, Chatsworth, Calif.) as recommended by the manufacturer. Plasmid pCDNA-FTIII is prepared by cloning HindIII and NotI-digested α1-3/4-fucosyltransferase cDNA (FTIII) obtained from the πH3M vector containing FTIII cDNA (Brian Seed, Massachusetts General Hospital, Boston, Mass.) was cloned into pCDNA3(neo) vector. FTIII fucosyltransferase is specific for both type 1 and 2 lactoseries oligosaccharide acceptor substrates and thus is capable of synthesizing both SA-Le^a and SA-LeX, respectively. The resulting cell line, B16F10FTIII, expresses SA-Le^a carbohydrate structure as demonstrated by flow cytometry analysis using MAb NS19-9 as compared to the parental B16F10, which did not show staining with this antibody.

The transfected cells were grown in the presence of G418 (500 μg/ml) (Gibco-BRL, Grand Island, N.Y.) for 10 days. To ensure the homogeneity of the transfected cells with respect to the expression of SA-Le^a, the cells were subjected to cell sorting using SA-Le^a specific MAb NS19-9 followed by FITC-conjugated goat anti-mouse immunoglobulin. The resulting cell line B16F10FTIII appeared to express SA-Le^a but not SA-LeX as assessed by FACS (not shown), suggesting that type 1 but not type 2 acceptors were available within the cells. Thus, the generated cell line made a suitable model to determine the role of SA-Le^a in the metastatic process since the tumor cells are devoid of SA-LeX. The tumorigenic dose for the C57B1/6 syngeneic tumor cells was established by i.v. injection of various numbers of cells. A 1×10^5 dose was chosen for further experiments as countable lung metastases were observed after i.v. injection of 1×10^5 of B16F10FTIII cells expressing SA-Le^a after 21 days.

The role of tumor cell adhesion to vascular EC via E-selectin and its ligand SA-Le^a interaction in metastasis formation was established in vivo in two ways. First, to directly assess the role of E-selectin in tumor colonization in vivo, the ability of B16F10 murine melanoma cells expressing SA-Le^a to colonize in the lung of E-selectin KO mice of C57B1/6 background [Staite, N. D. et al. 1998, *Blood*, 88: 2973–2979, and kindly provided by Dr. Daniel Bullard (University of Alabama at Birmingham, Ala.)] was determined in parallel with wild-type six- to 8-week-old C57B1/6 female mice (Jackson Laboratory, Bar Harbor, Me.). KO mice lack E-selectin expression.

B16F10FTIII cells positive for SA-Le^a were grown in vitro in Iscove's culture medium supplemented with 10% FBS for 1 week before injecting into mice. Cells were collected and washed twice in Iscove's medium without serum and suspended in PBS. One$\times10^5$ tumor cells in a volume of 200 μl in PBS were inoculated by intravenous (i.v.) route via tail vein. To test the effect of the peptide, animals were inoculated with a single dose of peptide at the time of tumor challenge. One mg of peptide was admixed with the tumor cells and together injected via i.v. route. Control animals received injection of tumor cells admixed with the same amount of unrelated peptide. Mice were euthanized after 3 weeks following tumor cells injection and lung and other organs were examined under dissecting microscope for the presence of tumor nodules. The lungs were excised and the number of nodules was enumerated for each animal without fixation of the lungs. Data were evaluated for statistical significance using a nonparametric unpaired two-tailed t test. The results are shown in FIG. 5.

Mice of both strains received i.v. injection of 1×10^5 B16F10FtIII tumor cells and mice were examined 3 weeks later. Only 20% of E-selectin deficient animals injected with tumor cells developed small numbers of lung metastasis while the rest of the E-selectin KO mice showed no detectable lung tumor nodules. Statistical analysis gave a P values <0.009 for E-selectin KO as compared to the control group (FIG. 4, A and C), respectively. Small nodules were observed in a few E-selectin KO mice that developed tumors whereas all animals in the control group developed multiple metastasis and some of them died earlier than 3 weeks. The results demonstrate that lung metastasis of tumor expressing SA-Le^a antigen is completely abrogated in most of the genetically manipulated mice that lack expression of E-selectin, highlighting the critical role of E-selectin in mediating carcinoma metastasis in vivo.

To further test the hypothesis that SA-Le^a expression supports adhesion of tumor cells to E-selectin on EC, the inhibitory effect of peptide mimicking SA-Le^a antigenic structure DLWDFVVGKPAG [SEQ ID NO: 63] on lung colonization of B16F10FTIII cells was tested. One$\times10^5$ tumor cells expressing SA-Le^a were admixed with a solution containing 1 mg of the peptide DLWDFVVGKPAG [SEQ ID NO: 63], followed by administration of the mixture to groups of mice. Because peptides in general show rather short half-life in mouse serum, in the peptide inhibition studies in vivo the peptide was admixed with the tumor cells to sustain the highest transient concentration of peptide at the time of tumor cell arrival into the lung capillary system. Animals were euthanized after 21 days following tumor challenge and the number of metastasis was enumerated in each lung whereas, no metastatic growth was detected in the liver.

Administration of the peptide DLWDFVVGKPAG [SEQ ID NO: 63] abrogated on average 50% lung colonization of the B16F10FTIII induced tumor nodules developed in control animals; some mice being completely devoid of tumor nodules (FIG. 4B). The injection of the peptide 1 hour prior to tumor cells did not influence the rate of metastases formation in comparison with the peptide administered together with tumor cells. Animals treated with peptide showed metastases ranging from 0 to 20 per lung (median 9.9), whereas, animals in the control group developed multiple tumor nodules with the number of metastases per mouse ranging from 3 to 40 per lung (median 20.7) (FIG. 4B and 4A), respectively). The difference was highly statistically significant ($p<0.008$). In addition, B16F10FtIII cells in C57B1/6 mice developed large tumor masses with diffused infiltration of tumor cells and some mice died before the termination of the experiment (median 16 days). Mice that developed metastases, despite treatment with SA-Le^a mimicking peptide, showed the presence of small tumor nodules and all survived the 3 week observation time.

These results suggest that the synthetic structural conformer mimicking SA-Le^a antigen is able to significantly block the adhesion of tumor cells to vascular endothelium at the early stages of the multistep process, thus reducing tumor metastases. This finding strongly suggests that the interaction of SA-Le^a carbohydrate tumor-associated antigen with E-selectin expressed on vascular EC is an important step in establishing tumor metastasis.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims to be construed to include all such embodiments and equivalent variations.

ERROR (15)

-continued

<210> SEQ ID NO 121
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: peptido-mimetic of a Lewis antigen

<400> SEQUENCE: 121

Ala Pro Trp Leu Tyr Gly Pro Ala
1 5

What is claimed is:

1. A peptide or polypeptide selected from the group consisting of: ASAVNLYIPTQE SEQ ID NO:84, VYLAPGRISRDY SEQ ID NO:85, VYLAPGRFSRDY SEQ ID NO:86, CTSHWGVLSQRR SEQ ID NO:87, RVLSPESYLGPS SEQ ID NO:88, VGNGVLMGRRG SEQ ID NO:90, RVLSPESYLGPA SEQ ID NO:92, GNCRYIGLRQPG SEQ ID NO:93, DIRVEPGGGYTH SEQ ID NO:94, APIHTYTGRARG SEQ ID NO:96, and RHTCVRSCGHDR SEQ ID NO:97 which binds to a selectin and blocks binding between said selectin and a Lewis SA-Le^a or SA-LeX antigen.

2. The peptide or polypeptide according to claim 1, which comprises a modification selected from the group consisting of (i) use of one or more D amino acids, (ii) insertion of a moiety which can provide a net positive charge at the N-terminus of said peptide or polypeptide, (iii) insertion of a spacer of greater than 3 amino acids interposed between the N- and C-termini to cyclize the peptide, (iv) insertion of a free hydroxyl on the C-terminus, (v) insertion of an amide or imide on the C-terminus, and (vi) insertion of a sequence of one or up to about 15 additional amino acids on the C-terminus.

3. A peptide or polypeptide selected from the group consisting of VGIWSVVSEGSR SEQ ID NO:102, RCSVGVPFTMES SEQ ID NO: 103, QDGVWEHVLEGG, SEQ ID NO: 104, DLWDWVVGKPAG SEQ ID NO: 1, VELSGRGGLCTW SEQ ID NO:105, VIGAASHDEDVD SEQ ID NO:106, TIEPVLAEMFMG SEQ ID NO:107, DKETFELGLFDR SEQ ID NO:108, FSGVRGVYESRT SEQ ID NO:109, PDDAPMHSTRVE SEQ ID NO:110, STGLMVDFLEPG SEQ ID NO:91, AKTFGLEHGCEA SEQ ID NO:95, GGTVEVWSIKGG SEQ ID NO:115, DHFSQAGSSNHH SEQ ID NO:116, DDPVTPVIDFGK SEQ ID NO:117, and RDGLIDFVVAGT SEQ ID NO:118 which binds to a selectin and blocks binding between said selectin and a Lewis SA-Le^a or SA-LeX antigen.

4. The peptide or polypeptide according to claim 3, which comprises a modification selected from the group consisting of (i) use of one or more D amino acids, (ii) insertion of a moiety which can provide a net positive charge at the N-terminus of said peptide or polypeptide, (iii) insertion of a spacer of greater than 3 amino acids interposed between the N- and C-terminus to cyclize the peptide, (iv) insertion of a free hydroxyl on the C-terminus, (v) insertion of an amide or imide on the C-terminus, and (vi) insertion of a sequence of one or up to about 15 additional amino acids on the C-terminus.

* * * * *

APR 24 2006

ERROR (1)

Exhibit AA

00270

PATENT TRADEMARK OFFICE

WST93AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	) Group Art Unit: 1632
Magdalena Blaszczyk-Thurin et al	) Examiner:
Appln. No. 09/831,047	)
Filed: May 3, 2001	)
For: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER	) May 9, 2002

Assistant Commissioner for Patents
Office of Initial Patent Examination
Customer Service Center
Washington, DC 20231

REQUEST FOR CORRECTION OF FILING RECEIPT

Sir:

Enclosed is a copy of the Filing Receipt received in the above-identified patent application. An error in the list of Applicant(s) for this application has been noted and correction to same is marked in red on the attached copy of the Filing Receipt (Exhibit A). Copies of the executed Declaration and Power of Attorney forms (Exhibit B) are attached to show the correct names of the Applicants. Further, Applicants and the undersigned attorney note that the Filing Receipt indicates that there are a total of (41) independent claims in this application. Copies of Applicants Fee Transmittal Letters (Exhibit C) are attached showing the total number of independent claims paid for in this application.

CERTIFICATE UNDER 37 CFR §1.8(a)

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: the Assistant Commissioner for Patents, Washington, DC 20231 on May 9, 2002.

Signature _____

Typed or printed name Debra N. Gerstemeier

Applicants respectfully request that the official filing receipt for this application be corrected to reflect the correct names of the Applicants as well as the correct number of independent claims.

The Director of the U. S. Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for the Applicants

By _____

Mary E. Bak
Registration No. 31,215
Spring House Corporate Center
Box 457
Spring House, PA 19477
Telephone: (215) 540-9206
Telefacsimile: (215) 540-5818

00270

PATENT TRADEMARK OFFICE PTO/SB/21 (08-00)
Approved for use through 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/831,047
Filing Date	05/05/2001
First Named Inventor	Magdalena Blaszczyk-Thurin et al
Group Art Unit	1632
Examiner Name	
Total Number of Pages in This Submission: 13	Attorney Docket Number: WST93AUSA

ENCLOSURES *(check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment / Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
  - ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Assignment Papers *(for an Application)*
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
- ☐ After Allowance Communication to Group
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to Group *(Appeal Notice, Brief, Reply Brief)*
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) *(please identify below)*:

2 pgs. Request for Correction of Filing Receipt w/Exhibits A, B, and C

Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	Mary E. Bak, Esquire Howson and Howson
Signature	Mary E. Bak
Date	May 9, 2002

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231 on this date: 05/09/2002

Typed or printed name	Debra N. Gerstemeier		
Signature	Debra N. Gerstemeier	Date	5-9-2002

PATENT

Serial No. 09/831047 Doc. No. WST93AUSA Atty/Sec MBS/lcly Date 5-9-2002

Inventor Malgorzata Bieszczyk-Duncan Client WST

Title Compositions and Methods for Treatment of Cancer

The following has been received in the U.S. Patent and Trademark Office on the date stamped hereon:

___ ___ pp. Spec., ___ Claims, ___ Abstract	___ ___ pp. Amendment: OA dtd ___
___ ___ pp. Declaration/Power of Attorney	___ ___ pp. Response: OA dtd ___
___ ___ pp. Verified Statement (Small Entry)	✓ 1 pp. Transmittal Letter
___ ___ shts. Informal Drawings	___ Issue Fee
___ ___ shts. Formal Drawings	___ Notice of Appeal & Fee
___ ___ pp. Assignment	___ Check # ___ for $ ___
___ ___ pp. Preliminary Amendment	✓ 21 Pages for Correction
___ ___ pp. Extension of Time	___ of Filing Receipt & Exhibits
___ ___ pp. Information Disclosure Statement with PTO-1449 and ___ references	___ A, B & C.

The Patent and Trademark Office is respectfully requested to place its stamp on this postal card and place it in the outgoing mail.

Respectfully,
HOWSON AND HOWSON

MBS

APR 20 2006

Exhibit BB

PATENT
Serial No. 09/831047 Doc. No. WST-13A00A Atty/Sec MBS/ly Date 5-9-2002
Inventor Maydelena Bleszczyk-Duran Client WST
Title Compositions and Methods for Treatment of Cancer
The following has been received in the U.S. Patent and Trademark Office on the date stamped hereon:

___ ___ pp. Spec., ___ Claims, ___ Abstract
___ ___ pp. Declaration/Power of Attorney
___ ___ pp. Verified Statement (Small Entity)
___ ___ shts. Informal Drawings
___ ___ shts. Formal Drawings
___ ___ pp. Assignment
___ ___ pp. Preliminary Amendment
___ ___ pp. Extension of Time
___ ___ pp. Information Disclosure Statement with PTO-1449 and ___ References
___ ___ pp. Amendment: OA dtd ___
___ ___ pp. Response: OA dtd ___
✓ 1 pp. Transmittal Letter
___ Issue Fee
___ Notice of Appeal & Fee
___ Check # ___ for $___
✓ 2 pg. Request for Correction
___ 4 pg. Copy of Exhibits A, B + C

1 RE JC59 MAY 22 2002 PATENT & TRADEMARK OFFICE

The Patent and Trademark Office is respectfully requested to place its stamp on this postal card and place it in the outgoing mail.

Respectfully,
HOWSON AND HOWSON
MBS

2006

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/831,047	05/03/2001	1646	814	WST93AUSA	5	41	10

CONFIRMATION NO. 8220

00270
HOWSON AND HOWSON
ONE SPRING HOUSE CORPORATION CENTER
BOX 457
321 NORRISTOWN ROAD
SPRING HOUSE, PA 19477

RECEIVED
MAY 07 2002

FILING RECEIPT

OC000000007961090

Date Mailed: 04/30/2002

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Filing Receipt Corrections, facsimile number 703-746-9195. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Magdalena Blaszczyk-Thurin, Philadelphia, PA;
Thomas Kieber-Emmons, Newtown Square, PA

Domestic Priority data as claimed by applicant

THIS APPLICATION IS A 371 OF PCT/US99/26277 11/05/1999
WHICH CLAIMS BENEFIT OF 60/107,478 11/06/1998

Foreign Applications

Projected Publication Date: Not Applicable, filed prior to November 29,2000

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Compositions and methods for treatment of cancer

Preliminary Class

Exhibit A
APR 20 2006

Exhibit DD


00270
PATENT TRADEMARK OFFICE

Attorney's Docket No.: WST93AUSA

TRANSMITTAL LETTER TO THE U.S. ELECTED OFFICE
(EO/US) - ENTRY INTO NATIONAL STAGE UNDER 35 USC 371

PCT/US99/26277	5 November 1999	6 November 1998
International Application No.	International Filing Date	Priority Date Claimed

COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER
Title of Invention

Magdalena Blaszczyk-Thurin and Thomas Kieber-Emmons
Applicant(s) for EO/US

Box PCT
Assistant Commissioner for Patents
Washington, DC 20231
Attn: EO/US

Sir:

Applicant herewith submits to the United States Elected Office (EO/US) the following items under 35 USC 371:

(1) This express request to immediately begin national examination procedures (35 USC 371(f)).

(2) A copy of the cover sheet for the published International Application along with a copy of the specification as filed: 106 pages, including 7 pages of claims, 5 sheets of drawings, 36 pages of Sequence Listing, and a copy of the 1 page International Search Report.

(3) a copy of the 5 page Request form.

(4) a first Preliminary Amendment for entry prior to calculation of the filing fees.

(5) our check in the amount of $796.00, covering the basic national fee as set forth in 37 CFR 1.492(a)(1) and based on the first Preliminary Amendment (39 total claims; 10 independent; and no multiple dependent).

Express Mail No. ET033649102US

Exhibit C

APR 20 2006

(6) A Second Preliminary Amendment.

(7) Our check in the amount of $18.00, covering the extra claim fees after entry of the second Preliminary Amendment (41 total claims; 10 independent; and no multiple dependent).

(8) Two (2) (4 pages) executed Combined Declaration and Power of Attorney forms.

(9) A 36 pages Sequence Listing (provided in specification).

(10) A 3.5" computer-readable diskette.

(11) A 1 page Statement under 37 CFR §1.821(f) and §1.825(a) and (b).

Copies of the following miscellaneous items are also enclosed:

(12) Copy of the 4 page Demand for International Preliminary Examination.

(13) Copy of the 4 page Written Opinion.

(14) Copy of the 5 page International Preliminary Examination Report.

Please charge any additional fees which may be required to effect entry into the National Phase and credit any overpayment to Deposit Account No. 08-3040.

Please direct all communications concerning this application to the undersigned.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for the Applicants

By Mary E. Bak

Mary E. Bak
Registration No. 31,215
Spring House Corporate Center
Box 457
Spring House, PA 19477
Telephone: (215) 540-9206
Telefacsimile: (215) 540-5818

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER, the specification of which is attached hereto and was filed as PCT International Patent Application No. PCT/US99/26277, on November 5, 1999.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed	Certified Copy Attached?	
(Number)	(Country)	(MM/DD/YYYY)		Yes	No

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

60/107,478	November 6, 1998
(Application Number)	(Filing Date, MM/DD/YYYY)

Exhibit B

I hereby appoint the following attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: STANLEY B. KITA, Registration No. 24,561; GEORGE A. SMITH, JR., Registration No. 24,442; WILSON OBERDORFER, Registration No. 17,379; MARY E. BAK, Registration No. 31,215, CATHY A. KODROFF, Registration Number 33,980, HENRY HANSEN, Registration No. 19,612, and WILLIAM BAK, Registration Number 37,277.

Address all telephone calls to Mary E. Bak at telephone no. (215) 540-9206. Address all correspondence to HOWSON AND HOWSON, Spring House Corporate Center, P. O. Box 457, Spring House, Pennsylvania 19477.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Magdalena Blaszczyk-Thurin

Inventor's signature _____ 3/29/01
Date

Residence: Philadelphia, Pennsylvania 19104
Citizenship: United States of America
Post Office Address: 28 University Mews, Philadelphia, Pennsylvania 19104

Full name of second inventor: Thomas Kieber-Emmons

Inventor's signature _____
Date

Residence: Newtown Square, Pennsylvania 19073
Citizenship: United States of America
Post Office Address: 3231 Saw Mill Road, Newtown Square, Pennsylvania 19073

TRANSMISSION VERIFICATION REPORT

TIME : 01/06/2005 10:54
NAME : HOWSON AND HOWSON
FAX : 2155405818
TEL : 2155409200
SER.# : BROD4J475241

DATE,TIME	01/06 10:51
FAX NO./NAME	17037469195
DURATION	00:03:19
PAGE(S)	14
RESULT	OK
MODE	STANDARD ECM

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	14

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Blaszczyk-Thurin et al.
Art Unit	1653
Examiner Name	S. Snedden
Attorney Docket Number	WST93AUSA

ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
- ☐ Fee Attached
- ☐ Amendment/Reply
- ☐ After Final
- ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
- ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
- ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please identify below):

3 pp. Second Request for Correction of Filing Receipt with 5 Exhibits

Remarks

Customer No. 00270

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON
Signature	

APR 20 2006

Auto-Reply Facsimile Transmission

TO: Fax Sender at 2155405818

Fax Information
Date Received: 1/6/2005 10:52:37 AM [Eastern Standard Time]
Total Pages: 14 (including cover page)

ADVISORY: This is an automatically generated return receipt confirmation of the facsimile transmission received by the Office. Please check to make sure that the number of pages listed as received in Total Pages above matches what was intended to be sent. Applicants are advised to retain this receipt in the unlikely event that proof of this facsimile transmission is necessary. Applicants are also advised to use the certificate of facsimile transmission procedures set forth in 37 CFR 1.8(a) and (b), 37 CFR 1.6(f). Trademark Applicants, also see the Trademark Manual of Examining Procedure (TMEP) section 306 et seq.

Received Cover Page ========>

01/06/2005 10:51 2155405818 HOWSON AND HOWSON PAGE 01/14

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM
(to be used for all correspondence after initial filing)

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Błaszczyk-Thurin et al.
Art Unit	1633
Examiner Name	J. Swanson
Attorney Docket Number	WST95AUSA

Total Number of Pages in This Submission: 14

ENCLOSURES (Check all that apply)

- ☐ Fee Transmittal Form
- ☐ Fee Attached
- ☐ Amendment/Reply
- ☐ After Final
- ☐ Affidavit/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
- ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
- ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please identify below):

3 pp. Second Request for Correction of Filing Receipt with 5 Exhibits

Remarks

Customer No. 00270

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON		
Signature	Mary E. Bak		
Printed name	Mary E. Bak		
Date	January 6, 2005	Reg. No.	31,215

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature	Kelly A. Kanstad		
Typed or printed name	Kelly A. Kanstad	Date	January 6, 2005

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PAGE 1/14 * RCVD AT 1/6/2005 10:52:37 AM [Eastern Standard Time] * SVR:USPTO-EFXRF-2/0 * DNIS:7469195 * CSID:2155405818 * DURATION (mm-ss):03-50

APR 20 2006

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	14

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Blaszczyk-Thurin et al.
Art Unit	1653
Examiner Name	S. Snedden
Attorney Docket Number	WST93AUSA

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation
- ☐ Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please Identify below):

3 pp. Second Request for Correction of Filing Receipt with 5 Exhibits

Remarks

Customer No. 00270

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON		
Signature	Mary E. Bak		
Printed name	Mary E. Bak		
Date	January 6, 2005	Reg. No.	31,215

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature	Kelly A Karstaedt		
Typed or printed name	Kelly A. Karstaedt	Date	January 6, 2005

WST93AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/831,047 Confirmation No.: 8220

Applicant : Blaszczyk-Thurin et al.

Filed : May 3, 2001

TC/A.U. : 1653 **URGENT!!**

Examiner : S. Snedden

Customer No. : 00270

Title : COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

LETTER

Sir:

This letter is being filed to resubmit a Request for Correction of Filing Receipt, which was timely filed at the USPTO on May 9, 2002 to correct errors in the number of Independent Claims and the listing of Applicant(s). This paper is being filed after receipt of the Notice of Allowance dated December 27, 2004, but before payment of the issue fee due March 27, 2005.

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this date: January 6, 2005
Signature Kelly A. Karstaedt
Name Kelly A. Karstaedt

Enclosed as support for the timely filing of the Request for Correction of Filing Receipt are the following:

(i) a two (2) page copy of the Request for Correction of Filing Receipt dated May 9, 2002 with accompanying transmittal papers (Exhibit AA);

(ii) a one (1) page copy of a Postcard stamped by the USPTO indicating receipt of item (i) on May 22, 2002 by the USPTO (Exhibit BB);

(v) a one (1) page copy of the Filing Receipt forwarded with the Request for Correction of Filing Receipt noting the errors in the number of independent claims and Applicant(s) (Exhibit CC);

(vi) a two (2) page copy of the Transmittal Letter, which was filed with the application on May 3, 2001, and correctly recites the total number of independent claims as "10" (Exhibit DD); and

(vi) a two (2) page copy of the Declaration, which was filed with the application on May 3, 2001, and correctly states the inventorship for this application as "Magdalena Blaszczyk-Thurin, Philadelphia, PA; Thomas Kieber-Emmons, Newtown Square, PA", and was forwarded with the Request for Correction of Filing Receipt (Exhibit EE).

Applicants respectfully assert that the above-indicated documents were timely filed at the United States Patent and Trademark Office in order to correct the following two errors in the Filing Receipt:

Error 1: the number of Independent Claims should be 10, not 41; and

Error 2: the listing of Applicant(s) should include Thomas Kieber-Emmons, Newtown Square, PA, so that the listing of Applicant(s) is correctly noted on the patent.

Applicants request correction of these two items.

The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to our Deposit Account Number 08-3040.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for Applicants

By Mary E. Bak

Mary E. Bak
Registration No. 31,215
Spring House Corporate
Center, Box 457
Spring House, PA 19477
Telephone: (215) 540-9200
Telefacsimile: (215) 540-5818

Customer No. 00270

PART B - FEE(S) TRANSMITTAL

ERROR (2)

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (703) 746-4000

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

00270 7590 12/27/2004

HOWSON AND HOWSON
ONE SPRING HOUSE CORPORATION CENTER
BOX 457
321 NORRISTOWN ROAD
SPRING HOUSE, PA 19477

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

Kelly A. Karstadt	(Depositor's name)
Kelly A. Karstadt	(Signature)
January 25, 2005	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/831,047	05/03/2001	Magdalena Blaszczyk-Thurin	WST93AUSA	8220

TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	YES	$700	$0	$700	03/28/2005

EXAMINER	ART UNIT	CLASS-SUBCLASS
SNEDDEN, SHERIDAN	1653	514-014000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 HOWSON AND HOWSON
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE	(B) RESIDENCE: (CITY and STATE OR COUNTRY)
The Wistar Institute of Anatomy and Biology	Philadelphia, Pennsylvania
The Trustees of the University of Pennsylvania	Philadelphia, Pennsylvania

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☑ Corporation or other private group entity ☐ Government

4a. The following fee(s) are enclosed:
- ☑ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☑ Advance Order - # of Copies 1

4b. Payment of Fee(s):
- ☑ A check in the amount of the fee(s) is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☑ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number 08-3040 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
- ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.
NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature Mary E. Bak Date Jan. 25, 2005

Typed or printed name Mary E. Bak Registration No. 31,215

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Approved for use th[illegible]h 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U[illegible]PARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no pe[illegible]is are required to respond to a collection of information unless it d[illegible]s a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission	2

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Blaszczyk-Thurin et al.
Art Unit	1653
Examiner Name	S. Snedden
Attorney Docket Number	WST93AUSA

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☐ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation
- ☐ Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please Identify below):
  1 pp. Issue Fee Transmittal

Remarks

Customer No. 00270

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	HOWSON AND HOWSON		
Signature	Mary E. Bak		
Printed name	Mary E. Bak		
Date	Jan 25, 2005	Reg. No.	31,215

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature	Kelly A. Karstaedt		
Typed or printed name	Kelly A. Karstaedt	Date	January 25, 2005

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

APR 26 2006

vivo. Indeed, SA-Lea specific monoclonal antibodies (MAbs) were inhibitory for adhesion of colon carcinoma cells to human umbilical cord vein endothelial cells (HUVEC).

In vivo studies have provided further evidence of the potential importance of the carbohydrate ligand/E-selectin interaction in tumor metastasis [Brodt *et al.*, 1997, *Int. J. Cancer* 71:612-619; Mannori *et al.*, 1997, *Am. J. Pathol.* 151:233-243 (Mannori II); Biancone *et al.*, 1996, *J. Exp. Med.* 183:581-587].

Alternatively, some carcinoma cells do not express these carbohydrate determinants (i.e., SA-LeX and SA-Le^a) and yet they can attach to EC prior to activation. Further, this adhesion is not augmented by cytokine treatment, suggesting E-selectin-independent adhesion [Iwai *et al.*, 1993, *Int. J. Cancer* 54:972-977; Tozeren *et al.*, 1995, *Int. J. Cancer* 60:426-431; Miyake *et al.*, 1992, *New Eng. J. Med.* 327:14-18; Garrigues *et al.*, 1992, *J. Cell. Biol.* 125:129-142].

Studies have also demonstrated the role of oligosaccharides in inflammatory responses. Neutrophil extravasation is enabled by a multistep process initiated by the selectin family [Kansas, 1996, *Blood* 88: 3259-3287]. Neutrophil-endothelial cell interaction mediated via the selectins in the context of vascular shear flow, are characterized by transient tethering of the neutrophils, followed by rolling of the neutrophil along the endothelial surface of the vessel wall. Studies *in vivo* and *in vitro* indicate that selectin-dependent neutrophil rolling is essential to subsequent events in the transmigration process. Neutrophils are exposed to endothelial cell derived IL-8, platelet-activating factor and other neutrophil-activating molecules [Lowe, 1997, In: The selectins: Inhibitors of leukocyte endothelial adhesion, pp. 143-177, Vestweber, ed., Harwood Academic Publishers, Reading, UK], which in turn promote activation of neutrophil P2 integrins, leading to integrin-dependent firm adhesion to the integrin receptor ICAM-1, and finally to neutrophil extravasation, possibly via homophilic interaction of platelet/endothelial cell adhesion molecule 1.

The expression of ligands for selectins, particularly E-selectin, by both neutrophils and carcinoma cells raises the possibility that metastases are equivalent to the inflammatory process in which tumor cells, particularly carcinoma cells, use the

MAb were incubated with increasing amounts of peptides and binding of free antibody to carbohydrate SA-Le^a was measured by enzyme linked immunosorbent assay. Results show competitive inhibition of MAb binding to solid phase SA-Le^a polyacrylamide matrix (SA-Le^a-PAA) by 12-mer peptides DLWDWVVGKPAG (■) [SEQ ID NO: 1] and DLWDEVVGKPAG (▲) [SEQ ID NO: 63] with respect to the MAb binding without peptide (100% of binding) and a negative control unrelated peptide (○).

Fig. 4 is a circular dichroism (CD) spectra comparing dodecapeptides DLWDWVVGKPAG (solid line) [SEQ ID NO: 1] and DLWDEVVGKPAG (---) [SEQ ID NO:63]. The spectra were recorded at 0.51 mg/ml for both peptides.

Fig. 5 is a graph illustrating the inhibition of lung experimental metastases with peptide DLWDEVVGKPAG [SEQ ID NO: 63]. Tumor cells were admixed with the specific or unrelated peptide solution (1 mg per mouse) and animals were inoculated with 1 X 10^5 B16F10FtIII tumor cells in 200 µl volume of PBS via tail vein. Results are from 4 experiments (5 mice in each group) are shown. Each dot represents enumerated tumor nodules in one lung in experimental group of C57Bl/6 mice treated with the peptide (*panel B*), control group of C57Bl/6 mice treated with unrelated peptide (*panel A*) and E-selectin knock-out (KO) mice of C57Bl/6 background (*panel C*). Statistical analysis using a nonparametric unpaired *t* test gave a two-tailed *p* values <0.008 and 0.009 for animals treated with peptide and E-selectin KO, respectively, as compared to control group. The horizontal bars represent median values and vertical bars denote standard deviation.

Detailed Description of the Invention

The invention is based on the discovery that peptido-mimetics of complex carbohydrate structures block adhesion of tumor cells and leukocytes to endothelial cells. The production of small peptide molecules which mimic complex carbohydrate structures are useful for blocking carbohydrate ligand-cell adhesion molecule interactions involved in metastasis, angiogenesis, and inflammatory responses. Thus, these peptido-mimetics are useful as anti-adhesion therapeutics. The evaluation of

VGNGVLMGRRG [SEQ ID NO:90], RVLSPESYLGPA [SEQ ID NO:92], GNCRYIGLRQFG [SEQ ID NO:93], DIRVEPGGGYTH [SEQ ID NO:94], APIHTYTGRARG [SEQ ID NO:96], and RHTCVRSCGHDR [SEQ ID NO:97].

Similarly, exemplary peptido-mimetics of SA-Le^a include, without limitation, VGIWSVVSEGSR [SEQ ID NO:102], RCSVGVPFTMES [SEQ ID NO:103] , QDGVWEHVLEGG, [SEQ ID NO:104], DLWDWVVGKPAG [SEQ ID NO:1], VELSGRGGLCTW [SEQ ID NO:105], VIGAASHDEDVD [SEQ ID NO:106], TIEPVLAEMFMG [SEQ ID NO:107], DKETFELGLFDR [SEQ ID NO:108], FSGVRGVYESRT [SEQ ID NO:109], PDDAPMHSTRVE [SEQ ID NO:110], STGLMVDFLEPG [SEQ ID NO: 91], AKTFGLEHGCEA [SEQ ID NO: 95], GGTVEVWSIKGG [SEQ ID NO: 115], DHFSQAGSSNHH [SEQ ID NO: 116]; DDPVTPVIDFGK [SEQ ID NO: 117], and RDGLIDFVVAGT [SEQ ID NO: 118].

As described in the examples below, families of mimics of carcinoma-associated antigens that represent SA-Le^a, in particular, were identified from a combinatorial peptide library using MAb NS19-9 specific for this carbohydrate structure. One of the peptides, DLWDWVVGKPAG [SEQ ID NO: 1], was selected that specifically competes for binding of MAb for SA-Le^a. This peptide displays an ability to partially inhibit neutrophil recruitment in an acute inflammation model *in vivo*. As described below, this peptide was analyzed by systematic amino acid replacements to identify optimal conformationally stabilized SA-Le^a mimics with higher affinity using a solid phase peptide array library. Comparison of signal intensities revealed significant differences in MAb binding as a result of substitutions, in particular at the N-terminus. Substitution analysis allowed for delineation of key residues that were sensitive to replacement. MAb NS19-9 discriminated against multiple amino acid substitutions affecting its recognition. Specific residues within this peptide were identified that may contribute to the mimicry of carbohydrate structure by the peptide.

On the other hand MAb NS19-9 could tolerate replacement of the lead peptide sequence by a variety of amino acids. These substitutions did not abrogate

ERROR (7)

databases for small molecular compounds include Cambridge Structural Database, Fine Chemical Database, and CONCORD database [for a review see Rusinko, A., Chem. Des. Auto. News, 8:44-47 (1993)].

Once suitable chemical entities or fragments have been selected, they can be assembled into a single compound. Assembly may proceed by visual inspection of the relationship of the fragments to each other on the three-dimensional image displayed on a computer screen in relation to the structure of the peptido-mimetic of this invention. Useful programs to aid one of skill in the art in connecting the individual chemical entities or fragments include the CAVEAT program [P. A. Bartlett et al, "CAVEAT: A Program to Facilitate the Structure-Derived Design of Biologically Active Molecules", in Molecular Recognition in Chemical and Biological Problems", Special Pub., Royal Chem. Soc. 78, pp. 182-196 (1989)], which is available from the University of California, Berkeley, CA; 3D Database systems such as MACCS-3D database (MDL Information Systems, San Leandro, CA) [see, e.g., Y. C. Martin, "3D Database Searching in Drug Design", J. Med. Chem., 35:2145-2154 (1992)]; and the HOOK program, available from Molecular Simulations, Burlington, MA.

Compounds that mimic a peptide of this invention or a ligand of the peptides may be designed as a whole or "*de novo*" using either an empty active site or optionally including some portion(s) of a known ligand(s). Suitable methods describing such methods include the LUDI program [H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. Comp. Aid. Molec. Design, 6:61-78 (1992)], available from Biosym Technologies, San Diego, CA; the LEGEND program [Y. Nishibata and A. Itai, Tetrahedron, 47:8985 (1991)], available from Molecular Simulations, Burlington, MA; and the LeapFrog program, available from Tripos Associates, St. Louis, MO. Other molecular modelling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al, "Molecular Modeling Software and Methods for Medicinal Chemistry", J. Med. Chem., 33:883-894 (1990). See also, M. A. Navia and M. A. Murcko, "The Use of Structural Information in Drug Design", Current

ERRORS (8), (9)

Opinions in Structural Biology, 2:202-210 (1992). For example, where the structures of test compounds are known, a model of the test compound may be superimposed over the model of the peptide of the invention. Numerous methods and techniques are known in the art for performing this step, any of which may be used. See, e.g., P.S. Farmer, Drug Design, Ariens, E.J., ed., Vol. 10, pp 119-143 (Academic Press, New York, 1980); U.S. Patent No. 5,331,573; U.S. Patent No. 5,500,807; C. Verlinde, Structure, 2:577-587 (1994); and I. D. Kuntz, Science, 257:1078-1082 (1992). The model building techniques and computer evaluation systems described herein are not a limitation on the present invention.

Thus, using these computer evaluation systems, a large number of compounds may be quickly and easily examined and expensive and lengthy biochemical testing avoided. Moreover, the need for actual syntheses of many compounds is effectively eliminated. Once identified by the modelling techniques, the proposed "new antibacterial" compound may be tested for bioactivity using standard techniques, such as the assays of the examples below.

The invention is further described in detail by reference to the following, experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

EXAMPLE 1: SCREENING OF A RANDOM PEPTIDE LIBRARY

To develop novel molecules to inhibit the adhesion of human adenocarcinoma cells to EC and, ultimately, to inhibit metastasis *in vivo*, peptides were derived from a 12-mer random peptide library. A diverse library of random dodecapeptides, displayed as flagellin-thioredoxin fusion proteins (FLITRX) on the surfaces of *E. coli* bacterial cells, was obtained from Invitrogen (Carlsbad, CA) [LaVallie *et al.*, 1993, *Bio/Technology*, 11:187-193]. This library enables efficient isolation of bacteria displaying peptides with affinity to immobilized antibodies or to other binding proteins.

very good. This peptide competes with LeY for BR55-2 binding. These data further demonstrate another method to develop peptide mimotopes that are specific. This method may be extended to identify motifs mimicking SA-LeX and SA-Le^a using the crystal structure of the lectin domain of E-selectin in optimizing respective mimotopes.

EXAMPLE 6: IDENTIFICATION OF SEQUENCES CRITICAL FOR MAB BINDING AND AN SA-Le^a MIMIC WITH HIGHER ANTIBODY BINDING AFFINITY

To analyze amino acid residues that are critical for NS19-9 recognition, an array library of 163 unique peptides was generated by systematic amino acid replacement in which each position of the starting peptide DLWDWVVGKPAG [SEQ ID NO: 1] was replaced by other L-amino acids. In addition, peptides were synthesized with simultaneous incorporation of multiple amino acids or with truncation of specific regions.

The peptide array of 163 unique peptides was generated by substituting all amino acids for each individual amino acid in lead peptide (DLWDWVVGKPAG [SEQ ID NO: 1] identified by combinatorial library panning with MAb NS19-9. An array of synthetic 12-mer peptides was synthesized using 90 X 130 mm polyethylene glycol-modified cellulose membrane functionalized with approximately 4 nmole/mm^2 amino groups, manufactured by Abimed (Lagenfeld, Germany). Standard Fmoc chemistry was used according to the manufacturer's instructions [Frank, R.,1992, *Tetrahedron 48*, 9217-9332]. The protected and activated amino acids were spotted using an Abimed ASP 422 robotic arm. All washing, dyeing and deprotection steps were done manually. The activated C-terminal amino acids were spotted leaving 10 mm space in each direction, at the concentration of 0.5 M in N-methyl pyrrolidone. A volume of 0.5 ml provides spot of 7-8 mm in diameter. Activation of the amino acids with dicyclohexyl-carbodiimide and N-hydroxy-benzotriazole was done 30 minutes before spotting. After each coupling cycle, the paper was washed with 12% acetic anhydride dissolved in N,N'-dimethylformamide (DMF) twice for a total of 10 min to endcap all unreacted amino groups. Repetitive removal of the Fmoc groups was

ERROR (11)

Table 4(cont'd)

Peptide Sequence	SEQ ID NO	Peptide Sequence	SEQ ID NO
EIHDWVVGKPAG	49	WDWVVGKPAG	56
DLWEHL	50	DWVVGKPAG	57
LDDDWVVGKPAG	51	WVVGKPAG	58
EIHEWVVGKPAG	52	VVGKPAG	59
DLWDHLLA	53	VGKPAG	60
LDDLWVVGKPAG	54	GKPAG	61
EIHEHLVGKPAG	55	KPAG	62

Comparison of signal intensities on the peptide array revealed that some substitutions led to enhanced NS19-9 binding allowing for identification of several peptides with increased binding affinity to the antibody (Table 5). Improvement of peptide binding was achieved mainly by substitution of residues 5 to 12 within the lead peptide, whereas no amino acid exchange at N-terminus (residues 1-4) led to the increased binding. The replacement of residues with amino acids containing polar groups such as Glu and Asp showed clearly an enhancing effect at the C-terminus but not the N-terminus.

Array analysis failed to reveal significant differences in the binding intensities between the peptides substituted at different positions, suggesting that single substitution at any position in this region with carboxyl groups can enhance the interaction with the MAb binding site. In addition, substitutions with Ile, Ala and Ser also improved MAb binding. Similarly, the simultaneous replacement of several residues with clusters of amino acids upstream from position 6 demonstrated enhanced binding. The highest intensity signal was, however, observed with peptide DLWDFVVGKPAG [SEQ ID NO:63] containing a single substitution at position 5 with Phe.

APR 20 2006

ERRORS (12) (13)

Table 5 lists the amino acid substitutions within peptide DLWDWVVGKPAG [SEQ ID NO: 1] that increase the binding of MAb NS19-9 using the peptide array.

Table 5

Peptide Sequence	SEQ ID NO	Peptide Sequence	SEQ ID NO
DLWDFVVGKPAG	63	DLWDWVVGKPDG	73
DLWDWVIGKPAG	64	DLWDWVVGKPAD	74
DLWDWVVAKPAG	65	DLWDWVKEKPAG	75
DLWDWVVSKPAG	66	DLWDWVLAKPAG	76
DLWDWVVEKPAG	67	DLWDWVVGEDAG	77
DLWDWVVDKPAG	68	DLWDWVVGKPEK	78
DLWDWVVGEPAG	69	DLWDWVKEEPAG	79
DLWDWVVGDPAG	70	DLWDWVVGKDEK	80
DLWDWVVGKEAG	71	DLWDWVVGEDEK	81
DLWDWVVGKDAG	72	DLWDWVKEEDEK	82

A distinct pattern of substitutions that led to increased or abolished signal intensities with respect to the C- and N-terminus suggests that the region close to the N-terminus might contribute to the specificity of the interaction with NS19-9. Amino acids close to the C-terminus appear to add significantly to the affinity of ligand binding.

EXAMPLE 7: INHIBITION OF BINDING OF MAb TO THE CARBOHYDRATE WITH SYNTHETIC PEPTIDES MIMICKING SA-Le^a STRUCTURE

DLWDWVVGKPAG [SEQ ID NO:1] and DLWDFVVGKPAG [SEQ ID NO:63] were chemically synthesized and tested for their ability to compete to immobilized synthetic SA-Le^a-PAA neoglycoprotein. To determine whether peptide SEQ ID NO: 1 and peptide SEQ ID NO: 63 are true mimics of SA-Le^a, dose-response experiments were carried out in order to determine the concentration of peptides

ERROR (14)

claims are intended to be construed to include all such embodiments and equivalent variations.

ERROR(15)

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/831,047
Filing Date	May 3, 2001
First Named Inventor	Blaszczyk-Thurin
Art Unit	1653
Examiner Name	S. Snedden
Attorney Docket Number	WST93AUSA

Total Number of Pages in This Submission: 7

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
  - ☐ Fee Attached
- ☑ Amendment/Reply
  - ☐ After Final
  - ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Reply to Missing Parts/ Incomplete Application
  - ☐ Reply to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
  - ☐ Landscape Table on CD
- ☐ After Allowance Communication to TC
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☐ Other Enclosure(s) (please identify below):

Remarks

Customer No. 00270

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Howson and Howson		
Signature	Mary E. Bak		
Printed name	Mary E. Bak		
Date	Dec. 6, 2004	Reg. No.	31,215

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature	Tracy U. Palovich		
Typed or printed name	Tracy U. Palovich	Date	12/6/2004

APR 20 2006

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	: 09/831,047	Confirmation No.: 8220
Applicant	: Blaszczyk-Thurin	
Filed	: May 3, 2001	
TC/A.U.	: 1653	
Examiner	: S. Snedden	
Customer No.	: 00270	
Title	: COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER	

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE AND AMENDMENT

Sir:

This paper is in timely response to the Office Action dated September 13, 2004. Please enter the following remarks and amendments.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

Certificate Under 37 CFR § 1.8

I hereby certify that this correspondence is being deposited with sufficient postage as first class mail with or is being facsimile transmitted to the United States Patent and Trademark Office at:
P.O. Box 1450
Alexandria, VA 22313-1450.
on 12/6/04
by Tracy O. Palovcik
Signature
Tracy O. Palovcik

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claims 1-5 (Canceled).

6(Currently Amended). ~~The~~ A peptide or polypeptide ~~according to claim 3 which is~~ selected from the group consisting of: ASAVNLYIPTQE SEQ ID NO:84, VYLAPGRISRDY SEQ ID NO:85, VYLAPGRFSRDY SEQ ID NO:86, CTSHWGVLSQRR SEQ ID NO:87, RVLSPESYLGPS SEQ ID NO:88, VGNGVLMGRRG SEQ ID NO:90, RVLSPESYLGPA SEQ ID NO:92, GNCRYIGLRQFG SEQ ID NO:93, DIRVEPGGGYTH SEQ ID NO:94, APIHTYTGRARG SEQ ID NO:96, and RHTCVRSCGHDR SEQ ID NO:97 which binds to a selectin and blocks binding between said selectin and a Lewis SA-Le^a or SA-LeX antigen.

Claim 7(Canceled).

8(Currently Amended). ~~The~~ A peptide or polypeptide ~~according to claim 3, wherein said Lewis antigen is SA-Le^a and said peptide or polypeptide is~~ selected from the group consisting of VGIWSVVSEGSR SEQ ID NO:102, RCSVGVPFTMES SEQ ID NO:103, QDGVWEHVLEGG, SEQ ID NO:104, DLWDWVVGKPAG SEQ ID NO:1, VELSGRGGLCTW SEQ ID NO:105, VIGAASHDEDVD SEQ ID NO:106, TIEPVLAEMFMG SEQ ID NO:107, DKETFELGLFDR SEQ ID NO:108, FSGVRGVYESRT SEQ ID NO:109, PDDAPMHSTRVE SEQ ID NO:110, STGLMVDFLEPG SEQ ID NO:91, AKTFGLEHGCEA SEQ ID NO:95,